criteria if they are to be capable of being used as probiotics; more particularly they must meet the criteria laid down by the Lactic Acid Bacteria Industrial Platform (LABIP; Guarner and Schaafsma, "Probiotics" *Int. J. Food Microbiol* 1998; 39:237-238; see Annex I) and others for the selection of probiotic microorganisms intended for use in humans. Reference is also made to Tannock (1997; TIBTECH 15: 270-274 "Probiotic properties of lactic acid bacteria: plenty of scope for fundamental R & D"), particularly at page 272 second column (see Annex II).

8. Arihara deals with a strain of *L. salivarius* which has been identified biochemically (using its fermentation profile) as a strain of *Lactobacillus salivarius* subsp. *salicinius*. *Lactobacillus salivarius* subsp. *salicinius* strain T140 was isolated from the "surface of Japanese pampas grass" which may have been contaminated by feces excreted by a domesticated animal. The Examiner states that these strains were isolated from the human gastro-intestinal tract. However, this is incorrect as no attempt was made to isolate lactic acid bacteria from washed and resected gastrointestinal tissue.

9. In contrast, the strains of *L salivarius* claimed in the present Application were deliberately isolated from the human GI tract (i.e. the environment in which they will be required to function) in order to ensure compliance with the recommended criteria laid down by the aforementioned LABIP. This is not the case

with the strain of *Lactobacillus salivarius* subsp. *salicinius* disclosed in Arihara *et al.*, nor is there any suggestion therein of such isolation.

10. Arihara *et al* describe 353 bacterial strains isolated from food, plants, saliva or animal feces. The Examiner takes the view that the cited reference discloses a *Lactobacillus salivarius* which appears to be identical to the presently claimed strain and refers for example to Table 1 on page 421. This is not the case for the reasons stated above. Furthermore, a number of the reported strain characteristics are different to those described by Collins *et al*. The assumptions made by the Examiner that the microorganisms disclosed in Arihara may be correct in that these bacterial strains may survive passage through the human gastrointestinal tract. However, it is incorrect to assume that these bacterial strains could exert any influence on the gastrointestinal microflora, or that these bacterial strains could interact with the human host resulting in certain health benefits. The fecal flora represents the luminal contents of the distal large bowel whereas the mucosa adhering microflora represent a highly specialised microenvironment. Adherent strains must be able to survive a more aerobic environment than that present in the lumen. In addition, adherent strains must survive and thrive in an immunologically hostile environment. These adherent bacteria must interact with the host immune system in order to survive and therefore will be immunomodulatory in nature.

14 The strains of *L. salivarius* claimed in the present Application have the ability to selectively kill pathogenic bacteria without killing off many closely related lactobacilli which have health--promoting properties and with which they exist in symbiotic relationship. *Lactobacillus salivarius* subsp. *salicinius* T140 differs from the presently claimed strains in not meeting the recommended criteria for the selection of probiotic strains proposed *inter alia* by LABIP.

15. This is an important trait as one would require a probiotic bacterium to antagonise the growth of pathogenic species but not affect the composition of the commensal flora. The isolation of *Lactobacillus salivarius* species from resected and washed human tissue resulted in the identification of strains with this trait. Environment pressures resulting in the selection of strains most suited to survive and thrive in that environment are obviously completely different between grass and the human gastrointestinal tract. Furthermore, salivacin 140, produced by the Arihara *et al.* strain, requires a high initial pH for production while the antimicrobial factors produced by the strains described in the present Application do not require such a high initial pH. These strain dependent differences demonstrate that the strains described in the present Application are novel and have not been previously described. In fact, Arihara *et al.* state "Thus salivacin 140 production is a strain-specific phenomena like most cases of the bacteriocin synthesis by lactic acid bacteria." (*sic*).

16. Ten Brink *et al.* disclose approximately 1000 lactobacillus strains isolated from fermented foods and feeds, human dental plaque and feces from laboratory animals and humans. The rationale underlying the isolation and screening programme described by ten Brink *et al.* is directed to the identification of lactic acid bacteria with anti-microbial properties suitable for use in food preservation. Thus, it was not suggested or anticipated by these authors that these isolates could be active within the human gastrointestinal tract by influencing pathogen adhesion or invasion. The strains of *L. salivarius* claimed in the present Application have been identified by means of biochemical and SDS-P AGE analysis as strains of *Lactobacillus salivarius* subsp. *Salivarius*. Thus, the respective strains are different. In fact, it is highly unlikely that these isolates would provide any health benefits to humans.

17. Two *Lactobacillus* strains are described in further detail in the ten Brink *et al.* reference. A *Lactobacillus salivarius* strain and a *Lactobacillus acidophilus* strain were reported to produce anti-microbial compounds designated salivaricin B and acidocin B, respectively. The *Lactobacillus acidophilus* strain is not considered further as it is a different species to those described by the present Application. *Lactobacillus salivarius* M7 produced salivaricin B which was active primarily against related lactobacilli. This is in contrast to the results described in the Application. In addition, salivaricin B is not heat stabile

while ABP118 is heat stable. Unlike the antimicrobial agent ABP 118 of the present Application, acidocin B produced by *Lactobacillus acidophilus* M46 of ten Brink *et al*. fails to retain any activity following the heat treatment at 121 °C. Under such conditions the antimicrobial agent ABP 118 retains at least 50% of its activity (see Table 9 of the specification of the present Application). The strains of *L. salivarius* claimed in respect of the present Application are identified *inter alia* by the fact that the secretory products produced thereby are maintained in the presence of physiological concentrations of human bile and human gastric juice. Thus, the strains are resistant to both bile and gastric acid, one of the recommended criteria of LABIP. Each of strains *Lactobacillus acidophilus* M46 and *Lactobacillus salivarius* M7 described in ten Brink was isolated from human dental plaque, not resected and washed human tissue, or grass near a barn as stated by the Examiner. The Examiner also states that these strains are likely to live within the human gastrointestinal tract. However, no studies were performed to assess the acid and bile tolerances of these strains. In fact, survival within the environment of dental plaque would suggest that these strains would not survive lower gastrointestinal tract transit. In addition, the bacteria associated with human dental plaque induce inflammatory responses. Adherent strains from resected and washed tissue would not be expected to induce inflammatory responses. Thus, the strains claimed in the present Application, and their anti-microbial factors, have not been previously described.